

Biological and botanical treatments as a dual control and biostimulation strategy for corn crops affected by *Fusarium verticillioides*

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Abstract

Phytopathogenic fungi in staple cereals can affect not only productivity but also the health of those who consume them. The objective of this research was to determine the antifungal activity of *Trichoderma harzianum* and *Bacillus subtilis*, as well as the hydroalcoholic extracts of *Larrea tridentata* and *Argemone mexicana* against *Fusarium verticillioides* under laboratory and field conditions. In vitro inhibition in dual cultures against the phytopathogen ranged from 82 to 86% for *T. harzianum*, *B. subtilis*, and *L. tridentata*; in contrast, for *A. mexicana*, it was 18%. The treatments reduced the incidence in the harvested grain by more than 70% in treated plants compared to the control. The application of treatments with *T. harzianum*, *B. subtilis*, *A. mexicana*, *L. tridentata* and a combination of *T. harzianum* and *B. subtilis* achieved a significant increase in plant height, which is directly related to the leaf area index; this resulted in optimal grain filling compared to the control; as a result, the yield potential of the Hernán Cortés hybrid was significantly expressed, with the botanical extracts outperforming the control and the microorganisms themselves, resulting in greater ear weight. Molecular corroboration of the phytopathogenic strain indicated 99% identity with *F. verticillioides* contained in the GenBank database.

Keywords:

Zea mays L., antagonists, biocontrol, biostimulation, incidence.



Introduction

Corn has great genetic diversity, resulting from centuries of human selection to adapt it to different environments or uses, allowing the generation of materials that meet both traditional and new needs (González-Santos *et al.*, 2023). Mexico is one of the largest consumers of corn in the world, as it is the basis of its diet. In addition, it is the most-produced crop in the country, making it the fourth-largest producer worldwide (González and Ávila, 2014).

Mexico produces 27 million tons (FIRA, 2024) and has a deficit of 11 million tons per year; the latter results from losses caused by pest infestations, mainly fungi, whose presence is favored by intense rainfall or drought (Peña, 2017). In regions of tropical and subtropical climates, the production of this cereal is seriously affected by *Fusarium verticillioides*, the agent responsible for rotting the stems, ears and roots of corn (Figueroa-López *et al.*, 2016).

Contamination of corn by *F. Verticillioides* extends beyond agronomic damage, making it a critical public and animal health challenge due to fumonisin synthesis. Recent studies underscore that these mycotoxins not only compromise the productivity and sustainability of the global agrifood chain (Hofstetter, 2025) but also act as potent neurotoxic and carcinogenic agents. In humans, chronic exposure has been linked to disruption of sphingolipid metabolism, increasing the risk of esophageal cancer and birth defects, including neural tube defects (Singh, 2025; Asiya *et al.*, 2026). Given this situation, rational control of the pathogen is imperative to mitigate the accumulation of these heat-stable toxins that persist in processed products.

This fungus is a plant pathogen transmitted through the soil, causing losses in grain quality (Einloft *et al.*, 2021). The commercial and sanitary qualities of cereals are important and determine the destination of the grain; in this context, colonization of cereals by *F. verticillioides* is frequently associated with the accumulation of fumonisins produced in the field or during storage, which poses a threat to their consumption as food for both people and livestock (Ferrigno *et al.*, 2023).

Developing a sustainable agricultural system that can meet the food demand of future generations requires integrating biological strategies that respect the environment and health (Jhariya *et al.*, 2019). The use of microorganisms, whether fungi such as *Trichoderma spp.* or bacteria such as *Bacillus spp.*, has been widely studied; although their inoculation has a positive effect on various crops, their combination could offer greater potential as growth promoters and biocontrol agents (Poveda, 2022).

In agriculture, these microorganisms can promote plant growth, improve tolerance to abiotic stresses and act as biological control agents, both directly and indirectly (Zin and Badaluddin, 2020). These microorganisms exhibit strong antagonistic activity against phytopathogens such as *Ralstonia solanacearum* and *Fusarium oxysporum*, under both laboratory and greenhouse conditions (Cao, 2018). Biological control also includes the use of plant-derived compounds, called plant extracts, which are characterized by their biodegradability and low toxicity (Hernández *et al.*, 2007).

Larrea tridentata, also called 'gobernadora', possesses active molecules with biocidal activity (Pañuelas *et al.*, 2015). Among these is a powerful antioxidant identified as nordihydroguaiaretic acid (NDGA), which has fungicidal properties (Arteaga *et al.*, 2005). *Argemone mexicana* or 'chicalote,' is an endemic Mexican species rich in alkaloids, terpenoids, flavonoids, phenolic compounds, long-chain aliphatic compounds and certain aromatic compounds, which are believed to generate antifungal activity (Soto, 2022).

Due to their biological activity against phytopathogenic fungi, secondary metabolites with fungicidal properties found in plants are considered a viable option for controlling these fungi (Madariaga-Mazón *et al.*, 2019). On the other hand, by altering essential and structural processes, these substances can promote plant growth, strengthen tolerance to abiotic stresses, and enhance grain yield and quality (Mrid, 2021).

The research expected that at least one of the used treatments would have an effective response against the phytopathogenic fungus *Fusarium verticillioides*, both in vitro and in the field. The study

aimed to determine the antifungal activity of *Trichoderma harzianum* and *Bacillus subtilis*, as well as the hydroalcoholic extracts of *Larrea tridentata* and *Argemone mexicana*, against *Fusarium verticillioides* under laboratory and field conditions.

Materials and methods

The research was conducted from June 2024 to November 2025 at the facilities of the Antonio Narro Autonomous Agrarian University (UAAAN), by its Spanish acronym, located in Saltillo, Coahuila, Mexico. The experimental site is located at coordinates 25° 21' 20.7" north latitude and 101° 01' 51.2" west longitude, at an altitude of 1 742 m. The climate is classified as Cfb according to Köppen. The average annual temperature is 16.4 °C, with an annual rainfall of 610 mm.

Collection of biological material

The Hernán Cortés hybrid corn seed was provided by the Mexican Corn Institute, located within the UAAAN facilities. It is a three-cross material with semi-dented white grain, adapted to altitudes of 1 000 to 1 800 m. This genotype has a maturity cycle of 160-170 days and a plant height of 2.8-3 m. It was specifically selected because it is in the release phase for the Mexican Bajío region and has previously shown susceptibility to *F. verticillioides*, making it imperative to develop biological and botanical control strategies to mitigate the impact of this pathogen. On the other hand, the *Bacillus subtilis* and *Trichoderma harzianum* strains were obtained from the microorganism collection of the phytopathology laboratory of the aforementioned university. For the botanical material, plants of *Larrea tridentata* and *Argemone mexicana* were collected in the locality of La Encantada, municipality of Saltillo, Coahuila.

Isolation and identification of the phytopathogenic fungus

The phytopathogenic fungus *F. verticillioides* was isolated from Hernán Cortés hybrid seed that was not chemically treated and exhibited rot symptoms. Based on its morphological and phenotypic characteristics, it was identified in PDA and Carnation Agar culture media (Leslie and Summerell, 2006).

Molecular verification of the strain

The *F. verticillioides* strain was cultured on PDA medium for 12 days and incubated at 26 °C. The extraction of genomic DNA and the sequencing of the ITS1 and ITS4 regions were performed by the laboratory of the San Luis Potosí Institute of Scientific and Technological Research (IPICYT), by its Spanish acronym. The sequences obtained were compared with those reported in the GenBank NCBI database.

Extract preparation

Extracts of *L. tridentata* and *A. mexicana* were obtained from leaf tissue collected in Coahuila, Mexico. The leaves were washed, disinfected and dried for subsequent pulverization. From each species, 49 g of plant material was taken and processed by constant magnetic stirring at room temperature (25 ±2 °C) with 70% methanol (v/v) for seven days. The macerate was then centrifuged in Falcon tubes and filtered to obtain the final crude extract.

In vitro inhibition capacity

Dual confrontation assays were established with ten replications, in which the degree of antagonism was determined using the scale of (Bell *et al.*, 1982) for *T. harzianum*. For *B. subtilis*, this measure was calculated using the equation: $PI = 100 - [(Gr \times 100) / Rp]$, where: PI= inhibition of fungal

growth; Gr= mycelial growth of the fungus; and Rp= radius of the plate (Hernández-Castillo *et al.* , 2008). In the case of *L. tridentata* and *A. mexicana* , tests were conducted with poisoned media in quintuplicate, where a biological window was opened and the concentrations of 750, 1 250, 1 500, 2 000, 2 500, 3 000, 3 500, 4 000, 4 500, 5 000, 5 500, 6 000 and 6 500 ppm were evaluated in order to determine the IC₉₀ of both extracts.

Field evaluation of biological effectiveness

The Hernán Cortés corn crop was grown under open-field conditions with natural pathogen infection in an area of 100 m². Rows were 6 m long, spaced 74 cm apart and plants were spaced 25 cm apart. To this end, the seed was inoculated one day in advance with the treatments, using carboxymethyl cellulose as a fixative and dye for identification. A second application was made with 15 ml of treatments when the plants were in the V2 stage, followed by a third application in the V4 stage (20 ml) directed at the base of the stem, and a fourth application in the anthesis stage by sprays directed at receptive stigmas.

The treatments evaluated were *T. harzianum* at a concentration of 1x10⁸ conidia ml⁻¹, *B. subtilis* at 1x10⁷ CFU, *A. mexicana* at an IC₉₀ of 13 000 ppm, *L. tridentata* at an IC₉₀ at 13 000 ppm, a combination of *T. harzianum* at 1x10⁸ conidia ml⁻¹ and *B. subtilis* at 1x10⁷ CFU and an absolute control. The experiment was set up in a randomized block design with four replications per treatment. The incidence of the disease was evaluated as a percentage (Rivas *et al.*, 2011).

Biological treatments in yield promotion

During the milk-dough grain stage, plant height was measured on five units per replication, for a total of 20 plants per treatment. At the end of physiological maturity, ears from all replications were harvested and weighed to determine yield.

Statistical analysis

The variables of in vitro inhibition, disease incidence, plant height, and ear weight were subjected to an analysis of variance. The comparison of means was performed using Tukey's test (p# 0.05) in SAS software (version 9.0).

Results and discussion

Strain isolation and identification

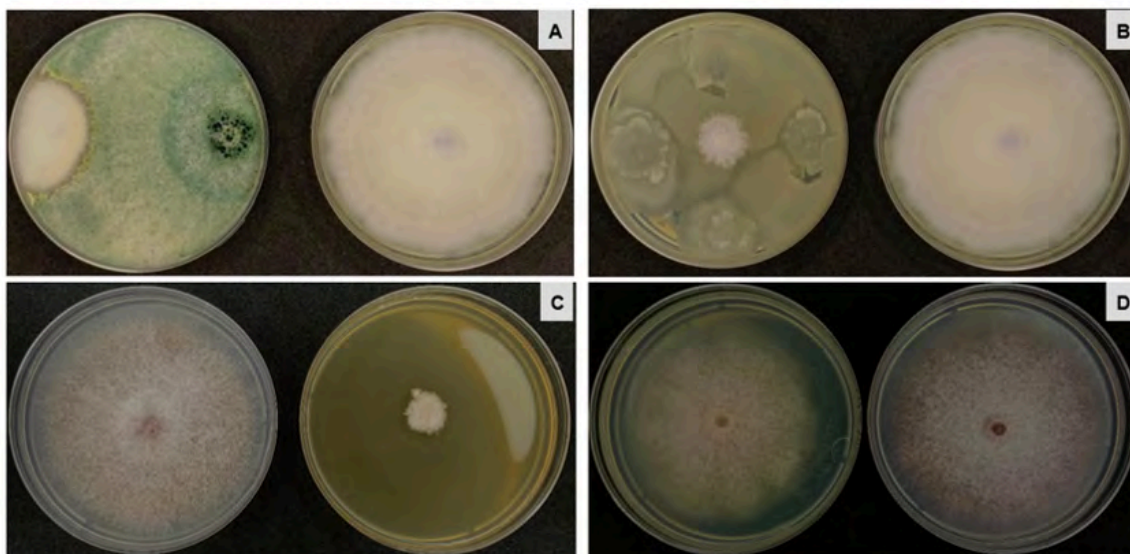
F. verticillioides was isolated from Hernán Cortés hybrid corn seed and was identified based on the formation of macroconidia, chain microconidia, and the absence of chlamydospores (Leslie and Summerell, 2006). Molecular corroboration showed a 99% match with *F. verticillioides* sequences contained in GenBank, identifying it as the causal agent of root and stem rot during vegetative stages, and of grain rot in the postharvest stage, which coincides with previous reports made by Gai *et al.* (2018); Czembor *et al.* (2019).

In vitro antagonistic capacity

The antifungal activity of the treatments is shown in Figure 1 , which depicts the growth of *F. verticillioides* in dual cultures evaluated at 11 days of incubation. Each panel shows the pathogen's growth under the influence of the antagonist or extract, contrasted with the control growth adjacent to it.



Figure 1. In vitro antagonism of *Trichoderma harzianum* (A); *Bacillus subtilis* (B); *Larrea tridentata* (C); *Argemone mexicana* (D); on the mycelial growth of *F. verticillioides* in dual cultures, evaluated at 11 days of incubation.



Radial growth inhibition differed significantly among treatments; the highest efficacy was obtained with *B. subtilis* (86%), followed by *T. harzianum* (82%) and *L. tridentata* extract (82%) at a concentration of 6 500 ppm; in contrast, *A. mexicana* exhibited the lowest inhibitory activity at 18% at 20 000 ppm. The antagonistic action of *B. subtilis* and *T. harzianum* is mainly related to their ability to compete effectively for space and nutrients in the pathogen's environment. In addition, they can synthesize various compounds with antimicrobial activity, as well as volatile metabolites.

They are also capable of producing hormones that modify plant physiology and activate systemic resistance mechanisms (Pedraza *et al.* , 2020). Alternatively, they can directly damage the pathogen through mycoparasitism, as in the case of *Trichoderma* (Elamathi *et al.* , 2018). Thus, the use of antagonistic microorganisms yields outstanding results, as those reported by Castro-del Ángel *et al.* (2020) .

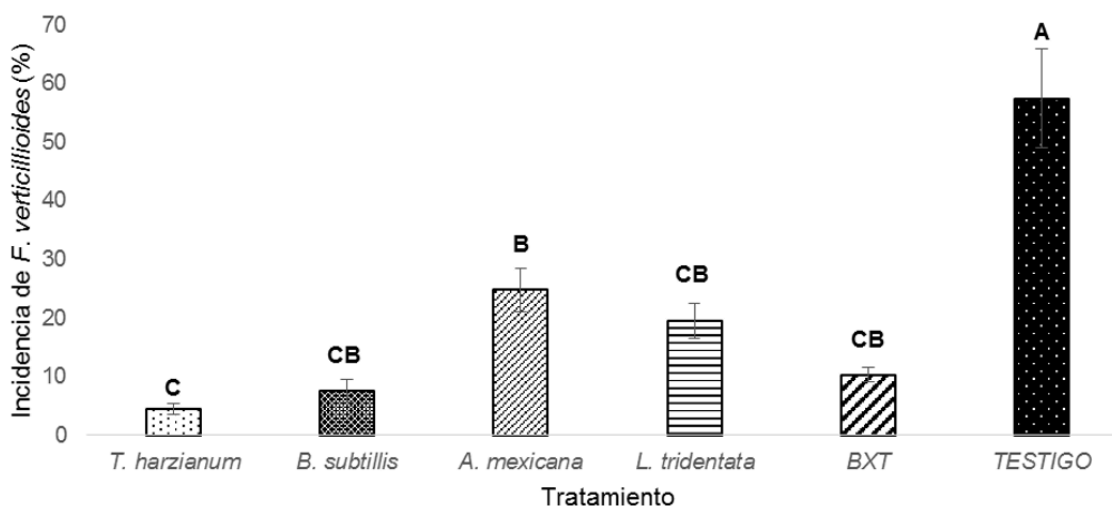
As for so-called botanical fungicides, numerous studies have shown that plant-derived phytochemicals have fungicidal effects. Plants can be considered perfect organisms with the potential to supply organic substances that can be classified as primary metabolites (proteins, carbohydrates, and fats) or secondary metabolites (terpenes, steroids, anthocyanins, anthraquinones, phenols, alkaloids, etc.) (Bahandari *et al.*, 2021).

Biocontrol effect in the field

Treatments with *T. harzianum* , *B. subtilis* , and *L. tridentata* significantly reduced the incidence of the phytopathogenic fungus ($p < 0.0001$). *T. harzianum* had a 5% incidence, whereas *B. subtilis* , *A. mexicana* and *L. tridentata* had an incidence of 8%, 25% and 10%, respectively, compared to the control (57%) (Figure 2).



Figure 2. Incidence of *F. verticillioides* in harvested grain of the Hernán Cortés genotype. The upper bars represent the standard deviation of the mean.



The susceptibility observed in the Hernán Cortés hybrid highlighted not only the risk of yield losses but also the potential accumulation of mycotoxins, such as fumonisins, produced by *F. verticillioides*. Reducing the incidence of the fungus by employing *T. harzianum* and *B. subtilis* (or *L. tridentata* extracts) represents an essential preventive measure to ensure food safety in highly productive yet vulnerable genetic materials.

The presence of *F. verticillioides* in corn crops not only implies a reduction in grain yield and quality but also carries the latent risk of accumulation of mycotoxins, particularly fumonisins (FB₁ and FB₂). These toxins are associated with serious pathologies in animals and are considered possible carcinogens to humans (Robledo Gutiérrez *et al.*, 2017).

In this context, the results obtained take on greater relevance, as the decrease in fungal incidence through the use of biocontrol agents and botanical extracts of *L. tridentata* suggests an indirect reduction in mycotoxin levels. This is critical for the Hernán Cortés genotype, whose susceptibility to this pathogen could compromise grain safety in the regions where it will be commercially released.

The efficacy observed in this work by using *T. harzianum* and *L. tridentata* extracts is not limited to suppressing mycelial growth but also projects a crucial benefit for grain safety. In this regard, Gong *et al.* (2024) point out that rational control using biological agents can inhibit the expression of genes responsible for fumonisin synthesis, which would explain how reduced fungal incidence translates into a lower toxic load.

For their part, the results align with those reported by Wang *et al.* (2024), who argue that using bioactive botanical compounds is an eco-friendly strategy capable of compromising the pathogen's integrity and mitigating the accumulation of FB₁. As stated by López-Nicora *et al.* (2025), integrating these preventive tools is the most effective way to ensure that corn complies with public and animal health standards, thereby avoiding the persistence of toxins that affect global food security.

In this regard, Castro-del Ángel *et al.* (2020) reported a treatment formulation using beneficial microorganisms capable of controlling *F. verticillioides*, achieving significant reductions in colonization; likewise, Sebayang *et al.* (2021) obtained similar results using bacteria of the genus *Bacillus* in combination with botanical extracts.

In addition to the above, it can be affirmed that the strains of the antagonists studied and the plant extracts are promising in reducing the incidence of *F. verticillioides*. The observed efficacy of biocontrol agents is partly attributed to the strains' origin. As these isolates are kept in the UAAAN's

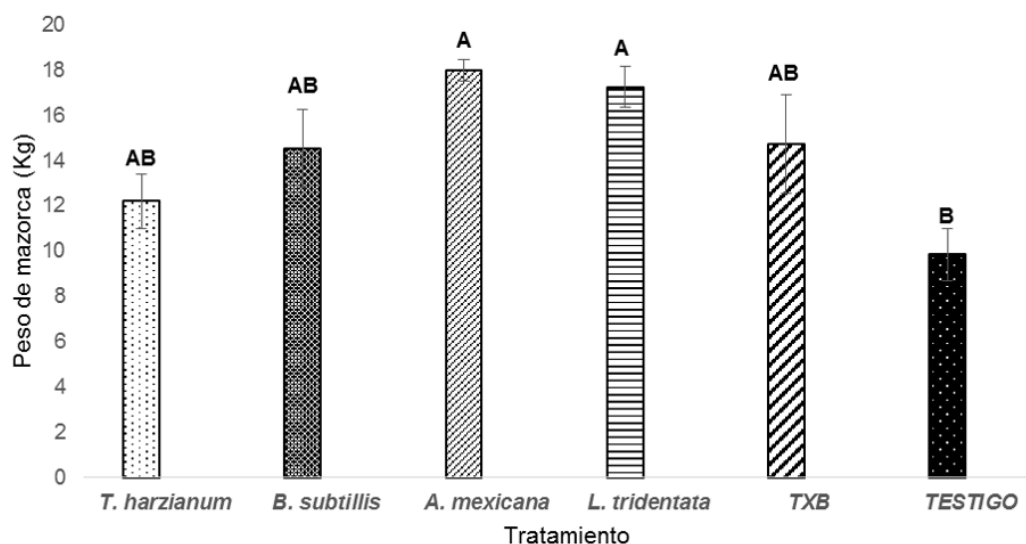
microorganism collection, they have been previously characterized, ensuring their viability and aggressiveness against soil phytopathogens.

The ability of *B. subtilis* and *T. harzianum* to reduce the incidence of *F. verticillioides* in the Hernán Cortés hybrid demonstrates that these microorganisms exhibit broad adaptive plasticity, which allowed their successful application in regions other than those of their original isolation, as in the areas evaluated for this new hybrid.

Biological treatments in yield promotion

The analysis of treatment activity against the phytopathogenic fungus *F. verticillioides* showed a significant increase in yield, a variable that is also directly affected by its presence, with higher weights compared to the control (Figure 3). It should be noted that the experiment was conducted free of chemical fertilization.

Figure 3. Ear weight in kg of the Hernán Cortés genotype with the treatments evaluated. The upper bars represent the standard deviation of the mean.



The leaf area index is extremely important for the plant because it determines the photosynthetic area and the accumulation of photosynthates (Mendoza *et al.* , 2017). The fixation of the treatments to the seed and post-emergence applications positively influenced the good development of leaf area and plant height, leading to more vigorous plants. The use of botanical treatments or species of the genus *Trichoderma* has the ability to visibly improve leaf area development, including the number of fruits in horticultural species or harvest yield (Hassan *et al.* , 2021).

On the other hand, the application of beneficial bacteria has been shown to improve growth in cereal crops such as wheat, with increased photosynthesis and water-use efficiency, which shows positive effects on aboveground biomass (Li *et al.* , 2022).

Among the benefits of antagonistic microorganisms, such as *T. harzianum* and *B. subtilis* , or plant extracts, such as those of *A. mexicana* and *L. tridentata* , is biostimulation, which favors plant growth; therefore, the plants subjected to the aforementioned treatments exhibited better development of the aboveground part, resulting in greater height (Table 1) and better ear weight compared with the control.

Table 1. Effect of biological treatments on the height of corn plants under natural infection of *F. verticillioides*.

Treatment	Plant height (m)	Standard deviation	Increase (%)
Control	2.39 c	0.054	-
<i>Trichoderma harzianum</i>	2.51 bc	0.047	16.3
<i>Bacillus subtilis</i>	2.59 abc	0.156	13
<i>Larrea tridentata</i>	2.7 ab	0.135	9.6
<i>Argemone mexicana</i>	2.78 a	0.01	8.4
<i>Bacillus</i> + <i>Trichoderma</i>	2.62 abc	0.12	5

Average values within a column followed by different lowercase letters differ significantly at $p \leq 0.05$ according to Tukey's honestly significant difference (HSD) test. The percentage increase was calculated using the control's average value as the reference.

Notable differences ($p = 0.0006$) were observed in the longitudinal development of corn plants in response to the different biological treatments following *F. verticillioides* infection. All the treatments evaluated exceeded the control in height, which had the lowest growth at 2.39 ± 0.054 m.

The treatment based on *A. mexicana* extract was the most effective, reaching a maximum height of 2.78 ± 0.01 m. This result not only represents the largest increase over the control (16.3%) but also shows the greatest experimental stability, as evidenced by the study's lowest standard deviation (0.01). On the other hand, the *L. tridentata* extract showed a similar behavior with a height of 2.7 ± 0.135 m.

Regarding microbial biological control agents, the *Bacillus* + *Trichoderma* consortium (2.62 ± 0.12 m) slightly exceeded the individual performance of *B. subtilis* (2.59 ± 0.156 m) and *T. harzianum* (2.51 ± 0.047 m). Notably, although treatments with *Bacillus* (alone and in consortium) increased height, they exhibited greater variability than plant extracts and the antagonistic fungus *T. harzianum*.

In this regard, the genus *Bacillus* shows biochemical properties, including phosphate solubilization, indoleacetic acid production and ACC deaminase activity, that are associated with plant growth promotion (Chávez-Ambríz *et al.*, 2016). As for the genus *Trichoderma*, this effect on the plant results from the activity of potent fungal signaling metabolites with hormone-like activity that are distributed in the soil, including indole compounds such as indole-3-acetic acid (IAA) and volatile organic compounds such as sesquiterpene isoprenoids and ethylene (Contreras-Cornejo *et al.*, 2024). In the case of plant extracts, growth stimulation has been linked to flavonoid content and antioxidant enzymes, providing a balanced nutrient supply for growth (Ei *et al.*, 2024).

Conclusions

F. verticillioides were isolated and identified as the causal agent of root, stem and ear rot in corn crops. Treatments with *T. harzianum*, *B. subtilis*, and *L. tridentata* showed high antagonistic activity against *F. verticillioides* in dual cultures during in vitro bioassays.

The incidence of *F. verticillioides* in the harvested grain was considerably reduced after the field bioassay with the treatments of *T. harzianum*, *B. subtilis*, *A. mexicana*, and *L. tridentata*, as well as the combination of *T. harzianum* with *B. subtilis*.

Treatments with *T. harzianum*, *B. subtilis*, *A. mexicana*, *L. tridentata* and the combination of *T. harzianum* with *B. subtilis* had a positive effect on the induction of growth and development of the corn crop and improved the expression of the yield potential of the Hernán Cortés hybrid, with the extracts of *A. mexicana* and *L. tridentata* being significantly superior.

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Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Article/Issue Information
Date received: 01 February 2026
Date accepted: 01 May 2026
Publication date: 01 August 2026
Volume: 17
Issue: 5
Electronic Location Identifier: e4136
DOI: 10.29312/remexca.v17i5.4136

Categories

Subject: Article

Keywords:

Keywords:

Zea mays L.
antagonists
biocontrol
biostimulation
incidence

Counts

Figures: 3
Tables: 1
Equations: 0
References: 41